Proteomics of Male Infertility

18

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Abstract

Screening of male infertility cases via clinical semen analysis is not apt to diagnose the causes in 30–50% of infertility cases. Such idiopathic cases with no known cause are difficult to monitor and thus provide momentum toward enhanced and sensitive diagnostic tools for infertility examinations. "Omics," the system biology approach to study the biological system on a large scale, includes proteomics as a newcomer. The era of clinical proteomics in combination with bioinformatics has emerged as a new tool to identify novel molecular markers for pathology. The supremacy of proteomics technology to characterize the proteome content of a cell or tissue on a large scale has enabled it to explicate both global and targeted proteins. Both seminal plasma and sperm serve to have the potential to be a preliminary material for identifying protein signatures related to infertility. The current chapter illustrates the lacunae allied with the clinical semen analysis for infertility investigations and exemplifies the role of clinical semen proteomics in male infertility identification.

Keywords

Sperm proteome • Seminal plasma proteome • Differentially expressed proteins • Male infertility

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Key Points

- Semen is rich in proteins, making it important to study their functional roles.
- Proteomics provides information beyond gene expression as proteins are the actual functional molecules.
- Sperm proteins are known to undergo posttranslational modifications, making proteomic studies important.
- Semen proteomic studies hold the potential for providing better platform for diagnosis of idiopathic male infertility.

18.1 Introduction

Clinical proteomics in combination with bioinformatics has emerged as a new tool to identify novel molecular markers for pathology. From the DNA sequence, one cannot extract information about the level of protein expression. Thus, the proteome which is the protein compliment of the genome is defined as the sum of all the protein species occuring during the lifetime of an individual, isoforms of the protein and the posttranslational modifications (PTMs) (Jungblut et al. 2008). The proteome fluctuates in response to the internal and external stimuli and undergoes disease-specific changes. Study of the differentially expressed sperm proteins that regulate fertilization holds potential in unraveling the molecular signatures related to male infertility. Transcriptome profiling of the sperm holds less potential for the post testicular investigations as sperm is both transcriptionally and translationally silent, thus PTMs play important role in inducing physiological changes responsible for fertilization.

Presently, the concept of seminal plasma (SP), as a biological fluid and a noninvasive clinical sample for urogenital diagnosis and for biomarker discovery of male reproductive disorders, is gaining attention. SP is an affluent and easily available source of protein identification owing to its high protein content of 35–55 mg/mL. As SP is a collection of fluids secreted from testis, epididymis, and other male accessory glands, it serves to be a reservoir of proteins crucial for sperm capacitation, sperm-zona pellucida interaction, and sperm-egg fusion (Tomar et al. 2012). SP is a possible target for early detection of male reproductive cancers (prostate and testicular), since proteins representative for cancer emerge earlier in SP than in blood serum (Drabovich et al. 2014).

In the current chapter, we first briefly discuss the risk factors associated with male infertility followed by the clinical tests available for its assessment and the lacunae allied with them followed by the importance of proteomics in infertility diagnosis. In the second part of the chapter, we broadly review the proteomics data of SP and sperm with special reference to male infertility.

18.2 Functional Tests for Male Fertility

Despite of physical examination, hormone analysis, and semen analysis, the etiology of male infertility in a large number of cases remains idiopathic. A number of normozoospemic patients appear for assisted reproduction due to failure of natural

conception for which the reasons are not obvious. Therefore, there is need to develop functional tests for male fertility, which can identify the lack of fertile (functional) sperm ejaculates with normal semen parameters according to the WHO criteria. It is not that we entirely lack the information about the potential functional analysis parameters; however, most of them suffer some or other limitations for bringing in regular clinical practice and are in need of further refinements. One such marker could be reactive oxygen species (ROS) level, which is well known to functionally impair spermatozoa. However, the short duration of activity of these molecules restricts the direct testing of ROS and its use as a method for infertility evaluation (Aitken et al. 1991). DNA fragmentation index (DFI) could be another such test; however, the lack of a standard DNA fragmentation test displaying universally agreed cutoff values limits its use as a diagnostic test for infertility assessment. The third could be the analysis of sperm/semen proteins for functional importance. The third aspect needs a lot of research on sperm proteomics, which has great potential in identifying the proteins of interest for this purpose.

18.3 Proteomics as a Diagnostic Tool for Evaluating Male Infertility

In the context of male infertility, clinical semen analysis providing a note for the concentration, motility, morphology of the sperm, and many other diagnostic tests still fail to screen the 30–40% infertility cases (Turek 2005). Thus, there is an unmet need for sensitive diagnostic tools for infertility investigations. With new scaling heights in molecular biology research, proteomics has expanded its horizon in sighting the pathological complexities of infertility and its causes. The protein biomarkers may help us toward better understanding of unknown causes of male infertility by dealing with the physiological functions of the proteins at tissue level that, in turn, can guide us to find better therapeutic solutions. It not only provides a platform to discover biomarkers of infertility but may also help in devising effective male contraceptives (Tomar et al. 2012; Upadhyay et al. 2013). By means of proteomic approaches, both global and targeted protein expression, regulation, and modification of proteins in various biological systems can be studied (Kolialexi et al. 2008). Several proteomic approaches are applied to study the proteome, its PTMs, and other different aspects that are directly linked with male fertility status. In the coming pages, a glimpse of the proteomic tools used to study the proteome will be discussed.

18.4 Proteomic Workflow for Sperm Characterization

The fundamental process of sperm proteome analysis comprises of purification of the sperm cells from the seminal fluid and making it free of contaminating cells (leukocytes, epithelial cells) and SP. Purity of the sperm cell preparation is a very decisive step and is the only step at the preliminary stage for the entry of minor contamination that could result in false-positive results. To circumvent this problem, density gradient centrifugation using Percoll or direct swim up method is used

for isolating sperm cells in humans (de Mateo et al. 2013). Another parameter to be considered in sperm proteomic study is to scrutinize the component of sperm to be concerned, target the entire cell or subcellular fractionation, and explore specific cell compartments. An advantage of subcellular proteomics is that it may allow the detection of low abundance proteins that may well escape detection in whole cell approaches (de Mateo et al. 2011).

Subsequent to the purification of sperm cells or the subcellular fractions, solubilized proteins are digested for peptide generation which is identified by Mass Spectrometry (MS). Essentially, two alternatives are there for MS identification of proteins: (1) separation of proteins followed by protein digestion and peptide identification and (2) generation of peptides by direct digestion of the proteins present in the crude mixture followed by their identification. Conventionally, proteins were separated using two-dimensional electrophoresis (2DE) followed by LC-MS-based protein identification. Low abundant proteins are tricky to detect via 2DE pertaining to its low sensitivity. With the advancements in proteomics field, 2DE was replaced with a more specific and sensitive technique of differential gel electrophoresis (DIGE), which labels the proteins with Cy dye and gives information about the differential expression of proteins. With these 2DE approaches, small number of proteins were elucidated, whereas LC-MS/MS-based studies depicted large number of proteins. The mass spectra generated by MS are evaluated using computer-based algorithm to determine whether peptides found in protein databases could produce spectra that resemble those observed experimentally.

To gain access about the biological information of the identified proteins, they are then categorically distributed using Gene Ontology (GO) database into three domains, cellular component, molecular function, and biological process. By and large, the outcome of a sperm proteomic study depends on (1) sample preparation, (2) protein extraction, (3) reduction of sample complexity, (4) optimum protein separation by advanced gel electrophoresis and/or LC, (5) MS protein identification with sufficient mass resolution and mass accuracy, (6) advanced computational analysis of peptide and protein data, (7) the bioinformatics analysis for the establishment of potential protein interactions and the clustering of molecular functions of newly identified proteins, and (8) essential verification analysis of proteomic data, using immunoblotting, biochemical assays, confocal microscopy, and/or functional testing (Holland and Ohlendieck 2015).

18.5 Whole Sperm Proteomics

In the past, 2DE was the method of choice to investigate the proteome of the sperm. The number of proteins distinguished in 2D maps ranged from 10 to 200 approximately. For the first time, Naaby-Hansen established a 2D map of the neutral and acidic human spermatozoa proteins using 2DE (Table 18.1). The study found 260 proteins ranging from 20 to 200 kDa and pI 4.5 and 7.8 (Naaby-Hansen 1990). In subsequent study by the same author and the colleagues, near about 1397 vectorially labeled sperm surface proteins belonging to membrane protein fractions were

Table 18.1 Sperm proteome studies

Pathological condition	Technique used	Major outcomes of the study	Function	Reference
Whole sperm proteome				
Normozoospermic	2D-PAGE	A total of 260 proteins ranging from 20 to 200 kDa and pI 4.5 and 7.8 were identified	Established a 2D map of the neutral and acidic human spermatozoa proteins using 2DE	Naaby-Hansen (1990)
Normozoospermic	2D-PAGE with nonequilibrium pH gradient electrophoresis (NEPHGE)	1397 sperm membrane protein were identified with novel isoforms of actin, beta-tubulin, pH 20, and several phosphotyrosine-containing proteins	Sperm membrane proteome	Naaby-Hansen et al. (1997)
Normozoospermic	LC-MS/MS	A total of 1760 proteins, with 1350 proteins corresponding to soluble fractions, 719 for insoluble fractions, and 309 for both soluble and insoluble fractions were identified		Johnston et al. (2005)
Normozoospermic	2D-PAGE and MALDI-TOF	A total of 98 proteins were identified	Major role in transcription, translation, protein turnover (23%), energy production (23%), metabolism (6%), cell cycle, apoptosis, and oxidative stress (10%)	Martínez-Heredia et al. (2006)
Normozoospermic	LC-MS/MS	A total of 1056 proteins were identified in Triton X-100 soluble and insoluble sperm fractions	Analysis of the metabolic proteome	Baker et al. (2007)
Normozoospermic	LC-MS/MS	A total of 4675 unique sperm proteins were identified	A total of 227 testis-specific proteins with different functions were characterized	Wang et al. (2013)

(continued)

Table 18.1 (continued)

Pathological condition	Technique used	Major outcomes of the study	Function	Reference
Subcellular fractions				
Normozoospermic (nuclei)	LC-MS/MS	A total of 403 proteins were identified	Zinc fingers and transcription factors were deduced for the first time which may be responsible for epigenetic marking and embryonic development. Histone proteins were the most abundant family	de Mateo et al. (2011)
Normozoospermic (tail)	LC-MS/MS	A total of 1049 proteins were identified	Proteins identified were related to sperm tail structure and motility (11%) and metabolism and energy production (26%). Metabolic proteome (24%) comprised of enzymes involved in lipid metabolism, including enzymes for mitochondrial beta-oxidation. Peroxisomal proteins were also a part of tail proteome	Amaral et al. (2013)
Normozoospermic (head and tail)	LC-MS/MS	A total of 1429 proteins were identified with 721 tail proteins and 521 head proteins	Proteases were localized in the head region, and structural and motility-related proteins were localized in sperm tail	Baker et al. (2013)
Sperm fibrous sheath	MS/MS	Unique ADP/ATP carrier protein, glycolytic enzymes, and sorbitol dehydrogenase were identified	Provides a clue that ATP is regulated independent of mitochondrial oxidation via the principal piece of the flagellum	Kim et al. (2007)
Sperm membrane proteins	MS and Edman degradation	Heat shock proteins were identified	HSP chaperones are accessible for surface labeling on human sperm	Naaby-Hansen and Herr (2010)
Comparative proteomics				
Fertile donors vs. failed fertilization at IVF	LC-MS/MS and MALDI-TOF/MS	20 differential proteins were identified	Two proteins, viz., secretory actin-binding protein and outer dense fiber protein 2/2, were overexpressed in the patient	Pixton et al. (2004)
Patients with normal fertilization vs. failed fertilization	2D-PAGE, LC-MS/MS, and MALDI-TOF/MS	12 differential proteins were identified	Proteins associated with gamete interaction, viz., the laminin receptor LR67 and the L-xylulose reductase were identified	Frapsauce et al. (2009)

Sperm of infertile patients vs. healthy fertile sperm	MALDI-TOF/MS	24 differential proteins were identified	Proteins belonged to different functional groups: sexual reproduction, metabolic process, response to wounding, cell growth, and/or maintenance. Proteins involved in cell communication, proliferation, and differentiation pathways were also identified	Xu et al. (2012)
Asthenozoospermic vs. normozoospermic	2D, MALDI-TOF/MS	10 differential proteins were identified	Energy metabolism enzymes, viz., isocitrate dehydrogenase subunit, carbonic anhydrase, were downregulated in low motility sperm, whereas phosphoglycerate mutase 2, triosephosphate isomerase, and glutamate oxaloacetate transaminase-1 were upregulated	Zhao et al. (2007)
Asthenozoospermic vs. normozoospermic	2D-PAGE, MALDI-TOF/MS	17 differential proteins were identified	Cytoskeletal actin-B, annexin-A5, cytochrome C oxidase-6B, histone H2A, PIP and precursor, calcium binding protein-S100A9 (2 spots), clusterin precursor, dihydrolipoamide dehydrogenase precursor, fumarate hydratase precursor, heat shock protein-HSPA2, inositol-1 monophosphatase, 3-mercapto-pyruvate sulfurtransferase/ dienoyl-CoA isomerase precursor, proteasome subunit-PSMB3, semenogelin 1 precursor, and testis-expressed sequence 12 were identified	Martínez-Heredia et al. (2008)
Asthenozoospermic vs. normozoospermic	2D-PAGE, MALDI-TOF/MS	12 differential proteins were identified	Phosphorylated forms of tubulin, reduced expression of gamma-tubulin were identified	Chan et al. (2009)
Asthenozoospermic vs. normozoospermic	2D-PAGE, MALDI-TOF/MS	8 differential proteins were identified	Proteins related to protein turnover, folding, and stress response proteins were identified	Siva et al. (2010)
Asthenozoospermic vs. normozoospermic	Nano UPLC-MS	66 differential phospho proteins were identified	Differentially expressed phosphorylated proteins were identified	Parte et al. (2012)

(continued)

Table 18.1 (continued)

Pathological condition	Technique used	Major outcomes of the study	Function	Reference
Asthenozoospermic vs. normozoospermic	2D-PAGE, MALDI-TOF/MS	16 differential proteins were identified	GRP78, lactoferrin, SPANXB, PGK2, flagellin, DJ-1, XPA binding protein 2, CAB2, GPX4, and GAPDH were the first to be identified as differentially expressed proteins in idiopathic asthenospermia patients	Shen et al. (2013)
Normozoospermic sperm samples with different IVF outcomes (pregnancy vs. no pregnancy)	TMT labeling, SDS-PAGE, LC-MS	66 differential proteins were identified	Proteins identified were involved in chromatin assembly and lipoprotein metabolism which have role in spermatogenesis	Azpiazu et al. (2014)
Asthenozoospermic vs. normozoospermic	TMT labeling, LC-MS/MS	80 differential proteins were identified	GO, cellular pathways, and clustering analyses indicated proteins associated with protein folding/degradation, vesicle trafficking, cytoskeleton, and energetic metabolism	Amaral et al. (2014)
Asthenozoospermic vs. normozoospermic	Label-free quantitative LC-MS/MS	127 differential proteins were identified	Functional category analyses indicated spermiogenesis, and motility-related proteins involved in that were related to metabolism, vesicle biogenesis cytoskeletal regulation, and protein degradation	Liu et al. (2015a)
Aged men and young asthenozoospermia patients	2D-PAGE, MALDI-TOF/MS	22 differential proteins were identified	Prostate and testis expressed 1 (PATE1) existed in both aged men and young asthenozoospermia patients. PATE1 was involved in sperm-egg penetration and sperm motility	Liu et al. (2015b)
Asthenozoospermic vs. normozoospermic (tail)	2D-PAGE, MALDI-TOF/MS	14 differential proteins were identified		Hashemitabar et al. (2015)

Globozoospermic vs. normozoospermic	2D-DIGE, MALDI-TOF/MS	35 differential proteins were identified	A total of nine proteins were found to be upregulated, and 26 proteins were found to be downregulated in round-headed spermatozoa. The differential proteins had important roles in spermatogenesis, cell skeleton, metabolism, and spermatozoa motility	Liao et al. (2009)
Oligoastheno zoospermic vs. normozoospermic	2D-DIGE, MALDI-TOF/MS	4 differential proteins were identified	Semenogelin II precursor and clusterin isoform 1 were not seen in the semen of infertile men	Thacker et al. (2011)
Functional proteomics				
Capacitated vs. non-capacitated normal sperm	2D-PAGE, MALDI-TOF/MS	Sperm phosphoproteome analysis was conducted for the first time revealing valosin-containing protein/p97 and two members of the A kinase-anchoring protein (AKAP) to be tyrosine phosphorylated during capacitation	Protein phosphorylation is the prerequisite for capacitation	Ficarro et al. (2003)
Capacitated vs. non-capacitated normal sperm	2D-PAGE, MALDI-TOF/MS	Proteins involved in energy metabolism (ATP synthase subunit alpha, L-asparaginase), flagellar organization (tubulin beta-2C chain, outer dense fiber protein, A-kinase anchor protein 4), protein turnover (heat shock-related 70 kDa protein 2) were downregulated after in vitro-induced capacitation seminal proteins such as clusterin and PIP were upregulated	Probably the motility apparatus of the capacitated sperm is deregulated, which may be induced by apoptosis-like mechanism	Secciani et al. (2009)

(continued)

Table 18.1 (continued)

Pathological condition	Technique used	Major outcomes of the study	Function	Reference
Capacitated normal sperm	Label-free phosphoproteomics	The activity of tyrosine phosphorylation kinase insulin growth factor 1 receptor (IGF1R) was augmented during sperm capacitation	Increased activity of tyrosine phosphorylation kinase IGF1R during sperm capacitation can be target for improvement in sperm functions in infertile men	Wang et al. (2015)
Impaired sperm-egg recognition vs. normal sperm	Label-free MS	Reduced expression of molecular chaperone, heat shock 70 kDa protein 2 (HSPA2)	Interaction analysis showed that HSPA2 was found in close association with two other proteins, sperm adhesion molecule 1 (SPAM1) and arylsulfatase A (ARSA)	Redgrove et al. (2012)
Acrosome reacted sperm	MS	Angiotensin-converting enzyme (ACE) and protein disulfide isomerase A6 (PDIA6) as the other new interacting partners of HSPA2	ACE and important component of the complex as its pharmacological inhibition significantly reduced the ability of human spermatozoa to undergo acrosome reaction	Bromfield et al. (2016)

catalogued using same 2DE technique (Naaby-Hansen et al. 1997). Additionally, the study revealed novel isoforms of actin, beta-tubulin, PH-20, and several phosphotyrosine-containing proteins in human sperm (Naaby-Hansen et al. 1997).

The low sensitivity issue associated with conventional 2DE approach hinders the detection of low abundant proteins. For this reason, researchers switched over to a more sensitive tool of MS. For the first time, extensive sperm proteome analysis of the soluble and insoluble sperm fractions was carried out using LC-MS/MS by Johnston and coauthors. Proteome analysis displayed about 1760 proteins in total, with 1350 proteins corresponding to soluble fractions, 719 for insoluble fractions, and 309 for both soluble and insoluble fractions. This sperm proteome characterization provides a physiologically relevant index of proteins (Johnston et al. 2005). In another study of its kind investigating the whole sperm proteome, 2DE separation was followed by matrix-assisted laser desorption/ionization time of flight-MS (MALDI-TOF/MS) identification of the proteins. The investigation revealed a total of 98 proteins with assigned functions. The proteins identified had major role in energy production (23%), transcription, translation, protein turnover (23%), cell cycle, apoptosis and oxidative stress (10%), and metabolism (6%). The functional details described in the present study paid impetus toward the better understanding of the sperm proteins (Martínez-Heredia et al. 2006).

Li et al. obtained a 2D reference map of 3872 proteins using narrow range pH strips and multiple 2D gels and identified the protein spots by MALDI-TOF/MS analysis, thus providing a comprehensive view of the sperm proteome, which can be useful in studying deregulations related to sperm infertility (Li et al. 2007). Similar to this, using comprehensive protocol of LC-MS/MS, the triton X-100 soluble and insoluble sperm fractions were analyzed, and 1056 different proteins were obtained (Baker et al. 2007). This is the first published list of identified proteins in human spermatozoa using LC-MS/MS analysis (Baker et al. 2007).

In a most extensive report, a total of 4675 unique proteins from human sperm have been successfully identified, of which 227 were testis specific. Furthermore, 500 proteins were annotated as drug targets, thus providing in-depth knowledge about the candidate targets for the development of male contraceptive drugs (Wang et al. 2013). In a recent study published by Amaral and her colleagues, the highest number of sperm proteins listed till date has been reported (Amaral et al. 2014). These proteins were reported to be involved in various functional pathways, such as metabolism, apoptosis, cell cycle, meiosis, and membrane trafficking. As discussed previously, functional annotations for the proteins identified are provided by using the GO catalogue.

18.6 Sperm Subcellular Proteomics

Subcellular proteomics helps in sorting different proteins from different compartments of the isolated sperm, viz., the head, tail, nucleus, and membrane proteins. As sperm is a cell with distinct sections having specific cellular roles, evaluation of the proteins from these compartments provides a clear view of the functioning of the sperm, events associated with fertilization and the proteins, which are responsible

for male infertility. Another relevance of studying the different fractions of the cell is the identification of the specific cellular localization of each protein and also the less abundant proteins. Extending this view, de Mateo and coauthors elucidated the sperm nuclear proteome highlighting some interesting facts not discussed before. Nuclear proteins are potentially relevant for epigenetic marking, proper fertilization, and embryo development. The study revealed a total of 403 proteins from the sperm nuclei, with histones as the most abundant family, zinc fingers, and transcription factors were deduced for the first time and may be responsible for epigenetic marking and embryonic development (de Mateo et al. 2011).

In another study reviewing the sperm tail proteins, a number of proteins were identified by LC-MS/MS and were found to be involved in metabolism and energy production, motility, and structure of the tail (Amaral et al. 2013). Interestingly, some peroxisomal proteins were also exposed in the investigation, thus paying momentum to the fact that both mitochondrial and peroxisomal pathways are active in the sperm and are imperative for the motility of the sperm (Amaral et al. 2014). Moreover, Baker and colleagues isolated and analyzed the proteome of sperm head and tail jointly from the same sperm sample, clearly pointing the compartmentalized expression of the head and tail proteins (Baker et al. 2013). For example, energy-providing proteins were found to be present in the tail, whereas the proteases were localized in the head region (Baker et al. 2013).

Similarly, some investigators have isolated the human sperm fibrous sheath, a cytoskeletal element unique to spermiogenesis. The proteomic analysis identified unique ADP/ATP carrier protein, glycolytic enzymes (reported for the first time), and sorbitol dehydrogenase in the fibrous sheath of the sperm (Kim et al. 2007). The presence of these proteins in the fibrous sheath provide a clue that ATP is regulated independent of mitochondrial oxidation via the principal piece of the flagellum (Kim et al. 2007). Highly heterogeneous structures of the sperm such as the head, mid-piece, and tail are enveloped under the sperm surface membrane. Throughout the epididymal transit and during initial events of fertilization (capacitation, zona binding, acrosomal reaction), the sperm membrane proteins experience complex remodeling.

Sperm membrane proteins are presumably entailed in fertilization process, critically sperm-oocyte interaction, and capacitation. By means of discrete enrichment techniques, several authors have analyzed membrane calcium binding proteins (Naaby-Hansen et al. 2010), heat shock proteins (Naaby-Hansen and Herr 2010), and membrane proteins with an affinity for zona pellucida (Nixon et al. 2015). As the membrane proteins have crucial role in the fertilization events, most of the studies focused toward categorization of surface antigens, which are involved in infertility and their use as immune contraceptives (Shetty et al. 2001; Bohring et al. 1999).

18.7 Comparative Proteomics of Anomalous Behavior of Sperm Proteins

Abnormal semen parameters are the most common cause of male infertility as suggested by the World Health Organization. Treatment of infertility using intra cytoplsmic sperm injection (ICSI) is a very effectual and routinely used procedure.

Nearly, 5% of in vitro fertilization (IVF) attempts have an unpredictable failure fate, regardless of normal sperm parameters. However, in the literature, there are accumulating evidences in humans that sperm defects such as defective zona binding or the zona-induced acrosome reaction count for 56% of total fertilization failure in assisted conception (Liu and Baker 2000, 2003). Being a heterogeneous phenotype, it is not necessary that these defects are the only underlying cause of reproductive failure, and thus other factors involved need to be assessed. The molecular nature of these defects is also needed to be traced, and for this several proteomics studies have been reported in the literature toward the potential identification of the sperm protein defects that might be responsible for failed fertilization at the IVF.

Using proteomics strategy, Pixton et al. compared the sperm proteome profile of the fertile donors with that of the patient who experienced failed fertilization at IVF inspite of normal semen parameters and found 20 consistent protein differences in the patient proteome profile (Pixton et al. 2004). Similarly, in another report, proteins associated with gamete interaction, viz., the laminin receptor LR67 and the L-xylulose reductase, have been found (Frapsauce et al. 2009). Xu and his group also focused on the major proteins extracted from infertile patients with normal semen parameters but failed IVF. The study revealed a total of 24 altered proteins, which were involved in energy production, structure and movement, and cell signaling and regulation (Xu et al. 2012). Abnormal morphology (globozoospermia), reduced motility (asthenozoospermia), and reduced number of sperms (oligo-/azoospermia) are other probable causes of male infertility, and comparative studies related to these pathological conditions at the proteome level have displayed a whole lot of proteins that are differentially expressed.

There are proteomic studies assessing differential expression pertaining to asthenozoospermic patients and normal fertile donors. For sperm motility, ATP production is of prime importance, and glycolysis and oxidative phosphorylation are the accepted pathways for ATP production in the mammalian sperm mitochondria. Enzymes associated with energy metabolism such as isocitrate dehydrogenase subunit, carbonic anhydrase, and glycolytic enzymes have been identified in a study (Zhao et al. 2007). Other proteins related to low sperm motility include Rho GDP-dissociation inhibitor 1 and outer dense fiber protein (sperm structural proteins) (Zhao et al. 2007), phosphorylated forms of tubulin, reduced expression of gamma-tubulin (Chan et al. 2009), various HSPs, disturbed cAMP-mediated protein kinase A signaling, and abnormal actin regulation (Parte et al. 2012), protein turnover, and folding and stress response proteins (proteasome alpha 3 subunit and heat shock-related 70 kDa protein 2) (Siva et al. 2010), which can be used for establishing biomarker signature for low sperm motility thus improving its diagnosis.

Recently, Hashemitabar and colleagues have isolated and compared the proteome of sperm tail fractions of asthenozoospermic semen samples with that of normal fertile donors using 2DE and MALDI-TOF MS/MS. The authors found differentially expressed proteins related to turnover, folding and stress response (proteasome alpha 3 subunit and heat shock-related 70 kDa protein 2), energy metabolism, sperm movement, stress response, signaling and transport, antioxidant activity, and structural proteins (Hashemitabar et al. 2015). Globozoospermia (round-headed spermatozoa with an absent acrosome) diagnosed by the presence of 100% round-headed spermatozoa on semen analysis is an aberrant nuclear

membrane and mid-piece defect making the patients with this condition absolutely infertile. Using DIGE coupled with MS over 61 protein spots were analyzed with nine proteins to be upregulated and 26 proteins to be downregulated in round-headed spermatozoa compared with normal spermatozoa. The differential proteins had important roles in a variety of cellular processes and structures, including spermatogenesis, cell skeleton, metabolism, and spermatozoa motility (Liao et al. 2009). Recently, Saraswat et al performed shotgun proteomic analysis (label free-LC- MS) of the sperm cells and seminal plasma proteins in normal and AS samples. The authors included 667 and 429 proteins for quantification in sperm and SP samples respectively. The investigators inferred that sperm motility pathway defects are reflected in sperm proteomic signatures and the seminal plasma data set does not imitate any of these defective pathways (Saraswat et al. 2017).

Oligoasthenozoospermia is a condition where both the sperm concentration and cellular motility are deranged posing the individual as infertile. Four unique proteins, semenogelin II precursor, prolactin-induced protein, clusterin isoform 1, and prostate-specific antigen (PSA) isoform 1 preproprotein were predominant in the semen of healthy men; however, semenogelin II precursor and clusterin isoform 1 were not seen in the semen of infertile men, suggesting unique differences in the spermatozoa protein profiles of fertile and infertile men (Thacker et al. 2011).

18.8 Functional Proteomics

The fate of fertilization is reliant on two hallmark events, viz., capacitation and acrosome reaction. Freshly ejaculated sperm goes through a number of functional modifications to accomplish fertilization proficiency. The process of acquiring the fertilizing potential starts by ejaculation and finally ends in the female reproductive tract. Austin and Chang in 1951 independently told that the sperm resides in the female tract to attain fertilizing capability and named it "capacitation" (Austin 1951, 1952; Chang 1951). Broadly, capacitation is defined as an ongoing process occurring during the sperm transport through female reproductive tract rendering sperm to undergo functional modifications, thus transforming it to competently fertile. The process is physiologically not complete until the spermatozoon reaches the oocyte (Bailey 2010). Once the sperm is competent, it binds to the zona pellucida, undergoes acrosome reaction followed by hyperactivated motility, and finally fuses with the oocyte (Bailey 2010).

Another major event succeeding capacitation is the acrosome reaction, which is calcium-dependent exocytosis triggered by the binding of the sperm to the oocytes zona pellucida (ZP) (Florman and Storey 1982). Outer acrosomal membrane fuses to the overlying plasma membrane at multiple points, thus liberating the entire contents, which pass through ZP and fuse with the oocyte plasma membrane. A prerequisite for this event is that the sperm should have undergone previous capacitation. Molecular mechanisms underlying capacitation and acrosome reaction are poorly understood. c-AMP-dependent tyrosine phosphorylation is a landmark for capacitation (Ficarro et al. 2003). Capacitated human sperm phosphoproteome analysis conducted for the first time revealed valosin-containing protein/p97 and two members of the A kinase-anchoring protein (AKAP) to be tyrosine phosphorylated during capacitation (Ficarro et al. 2003).

In another study of its kind, in vitro-induced capacitation proteome changes were illustrated. Altered proteome profile of normal versus capacitated sperm suggested that proteins involved in flagellar organization (tubulin beta-2C chain, outer dense fiber protein, A-kinase anchor protein 4), energy metabolism (ATP synthase subunit alpha, L-asparaginase), and protein turnover (heat shock-related 70 kDa protein 2) were downregulated after in vitro-induced capacitation (Secciani et al. 2009). Proteins that, instead, increased as a consequence of in vitro capacitation were seminal proteins such as clusterin and prolactin-inducible protein (PIP). The results indicate that the motility apparatus of the capacitated sperm is deregulated, which may probably be induced by apoptosis-like mechanism (Secciani et al. 2009). Label-free quantitative phosphoproteomics has been newly applied to investigate the overall phosphorylation events during sperm capacitation in humans and the phosphorylation sites involved. The results showed that the activity of insulin growth factor 1 receptor (IGF1R) tyrosine kinase is appreciably augmented during sperm capacitation posing it to be the target for improvement in sperm functions in infertile men (Wang et al. 2015).

The recognition and binding of spermatozoon to an ovulated oocyte is an imperative cellular event. Emerging evidences advocate for the concerted action of several sperm proteins for the accomplishment of sperm-egg fusion (Redgrove et al. 2011, 2012; Bromfield et al. 2016). Proteomic analysis of two such complexes using electrospray ionization mass spectrometry recognized the several components of the multimeric 20S proteasome and chaperonin-containing TCP-1 (CCT) complexes, with zona pellucida binding protein (ZPBP2) as a component of one of the complexes (Redgrove et al. 2011). Label-free MS-based comparative proteome analysis of sperm possessing an impaired capacity for sperm-egg recognition with normal cells revealed a reduced expression of the molecular chaperone and heat shock 70 kDa protein 2 (HSPA2) (Redgrove et al. 2012). Interaction analysis showed that HSPA2 was found in close association with two other proteins, sperm adhesion molecule 1 (SPAM1) and arylsulfatase A (ARSA), both of which have previously been implicated in sperm-egg interaction. The depletion of HSPA2 in the infertile patients posed impetus to the significance of this multimeric complex in arbitrating the sperm-egg contact thus paying attention to the male infertility causes (Redgrove et al. 2012).

Recently, Bromifield and colleagues have identified angiotensin-converting enzyme (ACE) and protein disulfide isomerase A6 (PDIA6) as the other new interacting partners of HSPA2, thus forming a multimeric protein complex participating in fertilization cascade. Moreover, the complex dwells in the membrane raft microdomains located in the peri-acrosomal region of the sperm head. Functional significance of the protein complex was assessed by inhibiting ACE, which significantly reduced the ability of human spermatozoa to undergo acrosome reaction (Bromfield et al. 2016).

18.9 Analysis of PTMs in Sperm Cells

In the rapidly changing environment persisting within the cell, the fragile homeostasis/balance is sustained by the proteins, which are the focal point of all the biological functions operative within the cell. The intricate process of transcription and translation (degradation), which govern the protein abundance, a composite

network of intra- and intermolecular interactions, PTMs (affecting protein activity and function) aid in adjusting to the dynamic alterations in the cellular environment. Usual aging, disease onset, and many other biological processes are the consequence of slight changes within this network. Mature spermatozoa are almost transcriptionally and translationally silent, and to attain its fertile destiny, it relies on PTMs that play important roles in sperm functions. Phosphorylation being the most commonly studied PTMs has been detected on approximately 17,500 proteins, and roughly one-third of the proteins in eukaryotic cell are phosphorylated at any time (Mann et al. 2002). Other recurrent PTMs are ubiquitination (~8100 proteins), glycosylation (~4500), lysine acetylation (~6700 proteins), and lysine methylation (~2400 proteins) (Pagel et al. 2015).

The molecular mechanisms underlying capacitation and acrosome reaction are poorly understood, and phosphorylation and glycosylation are the most prominent PTMs during these two processes. c-AMP-dependent tyrosine phosphorylation is a landmark for capacitation (Ficarro et al. 2003). Capacitated human sperm phosphoproteome analysis conducted for the first time revealed valosin-containing protein/p97 and two members of the A kinase-anchoring protein (AKAP) to be tyrosine phosphorylated during capacitation (Ficarro et al. 2003). Label-free quantitative phosphoproteomics has been newly applied to investigate the overall phosphorylation events during sperm capacitation in humans and the phosphorylation sites involved. The results showed that the activity of insulin growth factor 1 receptor (IGF1R) tyrosine kinase is appreciably augmented during sperm capacitation posing it to be the target for improvement in sperm functions in infertile men (Wang et al. 2015).

Recent reports state that sperm motility is coupled with α-tubulin acetylation (Bhagwat et al. 2014) based on the finding that protein acetylation can modulate proteasomal degradation of core histones and axonemal microtubule construction (Yu et al. 2015). Using proteomics approaches, global lysine acetylation profiles of normal uncapacitated sperm were characterized reporting 973 lysine-acetylated sites that matched to 456 human sperm proteins, including 671 novel lysine acetylation sites and 205 novel lysine-acetylated proteins. Another imperative discovery of the study was novel acetylation of voltage-dependent anion channel 2 at Lys-74 in the asthenozoospermic sperm cells (Yu et al. 2015). A number of proteins have been found acetylated at lysine residues in human capacitated sperm with functions in motility, capacitation, acrosome reaction, and sperm-egg interaction, thus proving to be an evidence for the importance of lysine acetylation in the sperm (Sun et al. 2014). O-linked or N-linked glycosylation is another PTM reported in the developing spermatozoa during the epididymal descent. The role of glycosylation in cell-cell recognition, adhesion, and recognition is well established, and in the sperm, it helps in gamete binding.

Using high-throughput glyco-FASP technique for the enrichment of glycopeptides and then subjecting to tandem MS analysis, 554 N-glycosylation sites and 297 N-glycosylated proteins in human sperm were identified (Wang et al. 2013). About 91% of the N-glycoproteins were either lysosomal, extracellular, or membrane proteins, and via in vitro fertilization assay, it was evident that glutathione peroxidase 4 (GPX4), a membrane glycoprotein, was effectively involved in gamete interactions (Wang et al. 2013). A recent study has stated that excessive sumoylation is a

marker of defective spermatozoa as some flagellar proteins, glycolytic and mitochondrial enzymes, and some heat shock proteins were found to be sumoylated at abnormally higher levels in nonmotile, two-tailed, microcephalic, and acephalic sperm (Vigodner et al. 2013). These sumoylated proteins were detected in the neck, flagella, and head regions as revealed by immunofluorescence and electron microscopy (Vigodner et al. 2013).

18.10 Seminal Plasma Proteomics in the Assessment of Male Fertility Status

As SP is a collective fluid derived from several organs and has protein constituents specific for the organ, therefore, differences in protein composition of SP might indicate an ongoing pathological process in a specific organ (Drabovich et al. 2014). For example, PSA, found at much higher concentrations in the semen than in the blood serum, is identified as a marker for prostatic diseases and is used for prostate cancer diagnosis. Proteome analysis of SP has raised the expectations for improved diagnosis and stratification of wide range of diseases (Davalieva et al. 2012). As SP is a collection of secretion of various tissue-specific proteins secreted by different male reproductive organs, it serves to be a potential source of protein biomarkers. SP proteome is subjected to alteration owing to male reproductive system disorders, thus leading to higher concentrations of organ-specific proteins, which can be quantified accurately by MS. Furthermore, it's proteome analysis could drive early diagnosis of testicular and prostate cancers as any cancer-specific protein appears much early in the SP than in the blood serum (Drabovich et al. 2014).

18.11 Seminal Plasma Proteome

The ejaculate is composed of 10% spermatozoa and 90% SP, with pH ranging from 7.2 to 8.0. SP serves to be the vehicle for the transport of spermatozoa during ejaculation from the male urethra thus escorting them to the female reproductive tract. Cell-free DNA, RNA, and microRNAs have also been identified in the SP, with microRNAs likely to be involved in spermatogenesis as their roles need to be further explored.

For the first time, SP proteins were electrophoretically separated in 1942 by a group of scientists (Gray and Huggins 1942; Ross et al. 1942), thus illustrating four protein components, α-globulin, β-globulin, γ-globulin, and albumin. Later advancements in the separation techniques resulted in the detection of nearly 40 proteins (Sensabaugh 1978). With the advent of new analytical paradigms in the field of electrophoretic protein separation through succeeding decades does the scientists were able to cut through the details of the SP proteome. In the early 1980s, Edwards and colleagues separated SP proteins by 2D-PAGE followed by blotting of proteins to nitrocellulose membrane and accordingly detected 200 proteins (Edwards et al. 1981).

The complexity of the SP was further attested by the introduction of high-throughput protein separation and identification tools, viz., soft ionization and MS.

In a study suggesting the role of SP proteins in impaired spermatogenesis, 750 proteins were identified including prostatic acid phosphatase (PAP), PSA, Zn-a-2-glycoprotein, glycodelin, and clusterin (Starita-Geribaldi et al. 2001). Furthermore, Fung et al. studied the SP proteome using LC-ESI thereby confirming the fact that the proteins were posttranslationally modified and that the multiple spots matching to the same parent protein were the isoforms of the same protein (Fung et al. 2004). Low molecular weight SP proteins of <30 kDa as truncated forms of semenogelin I and II, cystatin S, cystatin C, and variants of PIP were also identified.

In-depth analysis of SP proteome was conducted by Pilch and Mann in 2006, who catalogued a total of 932 proteins specific to each organ that has contributed to the formation of SP: seminal vesicle, prostate, epididymis, and Cowper's gland (Pilch and Mann 2006). Extracellular proteins secreted by the male sex glands, prostasomal proteins (originated from the epithelial lining of the prostate acini), and the proteins originated due to epithelial shredding were the three most prominent categories identified by the investigators. A large proportion of the proteome included proteins having functions in immunological reactions, providing metabolic sustainability and protection for the spermatozoa, involved in clot formation and liquefaction (Pilch and Mann 2006). Most recently, the investigators have used 2D liquid chromatography separations coupled to electrospray ionization and detection of mass spectra with Orbitrap™ and identified thousands of SP proteins. Largest library of SP proteins reported till date is of 3200 proteins in total as identified by Batruch et al. (2012).

Human SP contains a large array of proteins of clinical importance, and their characterization is imperative (Table 18.2). During fertilization process, the contact between the sperm and the egg is the decisive step for the future embryo to develop, and glycosaminoglycans (GAGs) have been reported to be vital for cell–cell interactions and communications. In the male reproductive biology, heparin, a GAG, is reported in processes, such as capacitation and acrosome reaction, and certain heparin-binding proteins (HBPs) interact with these GAGs present in the female reproductive tract, thus facilitating zona pellucida induction. Our group identified and characterized seven HBPs in the seminal fluid using affinity chromatography followed by MALDI-TOF/MS identification (Kumar et al. 2008). The major HBPs were semenogelin I fragment, semenogelin II, lactoferrin and its fragments, PSA, homolog of bovine SP proteins (BSP), zinc finger protein (Znf 169), and fibronectin fragments (Kumar et al. 2008).

As an extension to the abovementioned study, we also identified a group of concanavalin-A binding glycoproteins using affinity chromatography and subsequently identified them by MALDI-TOF/MS (Tomar et al. 2011). The major proteins identified in this study included aminopeptidase N, PSA, PAP, zinc-alpha-2-glycoprotein (ZAG), lactoferrin, Izumo sperm-egg fusion protein, progestogen-associated endometrial protein, and PIP (Tomar et al. 2011). Among the recent of all the studies done by our group, glycosylation sites, glycan compositions, and structures for 243 glycopeptides belonging to 73 N-glycosylation sites on 50 glycoproteins have been elucidated. Majority of the glycoproteins were complex type (83%) followed by high-mannose containing (10%) and hybrid type (7%), and most of the glycoproteins were either sialylated, fucosylated, or both (Saraswat et al. 2016).

Table 18.2 Seminal plasma proteome studies

Pathological condition	Technique used	Major outcomes of the study	Function	Reference
Whole SP proteome				
Normozoospermic	Fourier transform MS	A total of 932 proteins were identified	A large proportion of the proteome included proteins having functions in immunological reactions, providing metabolic sustainability and protection for the spermatozoa, involved in clot formation and liquefaction	Pilch and Mann (2006)
Normozoospermic	MudPIT-MS	A total of 3200 proteins were identified	Proteome included proteins having functions in immunological reactions, providing metabolic sustainability and protection for the spermatozoa, involved in clot formation and liquefaction	Batruch et al. (2012)
Normozoospermic	Affinity chromatography and MALDI-TOF	Major HBPs were semenogelin I fragment, semenogelin II, lactoferrin and its fragments, (PSA, homolog of bovine seminal plasma proteins (BSP), zinc finger protein (Znf 169), and fibronectin fragments	HBPs interact with the GAGs present in the female reproductive tract thus facilitating zona pellucida induction	Kumar et al. (2008)
SP proteome (concanavalin-A binding glycoproteins)	Affinity chromatography and MALDI-TOF		The major proteins identified in this study included aminopeptidase N, PSA, PAP, ZAG, lactoferrin, Izumo sperm-egg fusion protein, progestogen-associated endometrial protein, and PIP	Tomar et al. (2011)

(continued)

Table 18.2 (continued)

Pathological condition	Technique used	Major outcomes of the study	Function	Reference
SP glycoproteome	Affinity chromatography, MS/MS	Glycosylation sites, glycan compositions, and structures for 243 glycopeptides belonging to 73 N-glycosylation sites on 50 glycoproteins have been elucidated	Glycoproteins are involved in sperm and egg interaction	Saraswat et al. (2016)
Comparative proteomics				
Azoospermic vs. normozoospermic	MS	Nearly 700 proteins with acid phosphatase, PSA, Zn-a-2-glycoprotein, glycodelin, and clusterin were identified	Impaired spermatogenesis	Starita-Geribaldi et al. (2001)
Azoospermic vs. normozoospermic	2D-DIGE, MS/MS	STAB2, CP135, GNRP, and PIP as the potential markers		Yamakawa et al. (2007)
Nonobstructive Azoospermia vs. normozoospermic	MS/MS	28 differential proteins identified		Bai et al. (2007)
Asthenozoospermic vs. normozoospermic	LC-MS/MS	100 differential proteins identified	Epididymal secretory protein E1 and epididymal secretory protein E4 were increased in asthenozoospermic SP thus pointing toward the functional abnormalities in the prostate and epididymis contributing to abnormal sperm motility. Also downregulation of DJ-1 protein, involved in regulating oxidative stress concluded that increased levels of reactive oxygen species due to deregulated DJ-1	Wang et al. (2009)
Normal vs. asthenozoospermic, oligozoospermic, azoospermic SP	2D-DIGE, LC-MS/MS	8 proteins were differentially expressed in Azoospermia group	Fibronectin, PAP, proteasome subunit alpha type-3, beta-2-microglobulin, galectin-3-binding protein, PIP, and cytosolic nonspecific dipeptidase	Davalieva et al. (2012)

Normal vs. globozoospermic, oligozoospermic, SP	LC-MS/MS	20 proteins were differentially expressed	The functional analysis identified biological regulation as the major processes affected and determined that most of the identified proteins were of extracellular origin	Sharma et al. (2013a)
Infertile men vs. healthy donors to identify oxidative stress-related proteins	LC-MS/MS	14 differential proteins identified	PIP was found to be more abundantly present in men with increased levels of ROS. Gene ontology annotations showed extracellular distribution of proteins with a major role in antioxidative activity and regulatory processes	Sharma et al. (2013b)
Oligoasthenoteratozoospermia vs. normozoospermic	LC-MS/MS	Tubulin-folding cofactor B, alpha-1-antichymotrypsin, aldose reductase		Herwig et al. (2013)
Oligoasthenozoospermic vs. normal SP	2D-DIGE, LC-ESI/MS	4 differential proteins were identified	Epididymal secretory protein E1 and galectin-3-binding protein were under-expressed and lipocalin-1 and PIP	Giacomini et al. (2015)

The accuracy and proficiency of intracellular signaling pathways is under the influence of multiprotein complexes. Our group for the first time reported that ZAG is present as complex with PIP (Hassan et al. 2008). Human serum albumin (HSA), known to preserve in sperm motility, is proposed by our group as another plausible interacting partner of PIP (Kumar et al. 2012). Additionally, Tomar et al. identified the other interacting partners of PIP by co-immunoprecipitation followed by MS, suggesting semenogelin 1 fragments binding with PIP, thus providing its link in aiding spermatozoa to acquire motility (Tomar et al. 2013).

Later we also purified and characterized a zinc-binding high molecular weight multi-protein complex from human SP. The complex contained isoforms/fragments of different proteins with PSA, ZAG, PAP, and PIP as the major proteins of this complex (Yadav et al. 2011). Among the low molecular weight proteins, our group purified three cystatins (cysteine proteinase inhibitors), viz., cystatin 9, cystatin SN, and SAP-1 (N-terminal truncated form of cystatin S), and studied their enzyme kinetics (Yadav et al. 2013). Further interaction studies conducted on SAP-1 and heparin concluded that SAP-1 interacts with heparin and the binding is dependent on the chain length of heparin (Yadav et al. 2015).

Prostasomes, the membrane-enveloped vesicles secreted by the epithelial lining of the prostate acini, are rich source of intracellular proteins and are important for spermatozoa survival. Utleg et al. studied the composition of these prostasomes using LC-MS/MS and reported 139 proteins including enzymes, structural proteins, GTP binding proteins, and transport proteins. More importantly, majority of the proteins were secreted by the prostate (Utleg et al. 2003). Apart from focusing on the protein components of SP, researchers have also investigated the peptide constituents of the SP (Kausler and Spiteller 1992; Goverde et al. 1998; O'Mahony et al. 2000).

18.12 Comparative SP Proteomics with Clinical Objectives

From clinical viewpoint, the relevance of SP proteomics lays in the identification of male infertility-associated biomarkers. As SP constitutes the 90% of the total semen volume with the rest 10% engaged by spermatozoa and also higher concentration of tissue-specific proteins are present in it, it is a probable source of protein biomarkers. Extensive literature is there dealing with the identification of SP constituents; however, studies having inclination towards male infertility with extensive comparative analysis of SP proteome providing a correlation between SP proteins and male infertility are meager. In the first study of its kind, Starita-Geribaldi and colleagues studied the SP proteome in impaired spermatogenesis and compared the proteome from fertile men with vasectomized or azoospermic men and revealed nearly 700 proteins including acid phosphatase, PSA, ZAG, glycodelin, and clusterin (Starita-Geribaldi et al. 2001).

Most commonly, male infertility is diagnosed by a laboratory-based semen analysis for the presence of spermatozoa in the seminal fluid. Azoospermia, a condition with absence of sperm in the semen, is the most severe form of male infertility

(Jarow et al. 1989). Major attempt in this direction was made by Yamakawa et al. who analyzed the differential expression of proteins with respect to azoospermia condition and displayed stabilin 2 (STAB2), 135 kD, centrosomal protein (CP135), guanine nucleotide, releasing protein (GNRP), and PIP as the potential markers (Yamakawa et al. 2007). In another report, SP of nonobstructive azoospermia patients and healthy fertile males were compared showing 28 differentially expressed proteins (Bai et al. 2007). Our group purified PIP by immunoprecipitation and quantified its level in azoospermic SP samples using ELISA kit and found no significant change in its concentration in normozoospermia and oligozoospermia, while its expression was downregulated in azoospermia, thus paying impetus to the above findings of Yamakawa indicating PIP to be a plausible marker of azoospermia (Tomar et al. 2012).

Comparative proteomic analysis of normal and asthenozoospermic SP proteomes revealed 741 proteins, most of which were of epididymal and prostate origin. Moreover, epididymal secretory protein E1 and epididymal secretory protein E4 were increased in asthenozoospermic SP, thus pointing toward the functional abnormalities in the prostate and epididymis contributing to abnormal sperm motility (Wang et al. 2009). Another crucial finding of the study was the downregulation of DJ-1 protein, which is involved in regulating oxidative stress thus concluding that increased levels of reactive oxygen species due to deregulated DJ-1is an indicator of poor semen quality (Wang et al. 2009). High-resolution multidimensional protein identification technology (MudPIT) analyzed the SP proteome of normal and postvasectomy (PV) data sets, thus reporting 32 proteins unique to controls and three unique to PV patients (Batruch et al. 2011).

The same authors recently catalogued more than 2000 proteins in nonobstructive azoospermia (NOA) subjects. Some of the proteins identified in this study, viz., LDHC, ELSPBP1, CES7, A2M, OVCH2, PTGDS, GPR64, and ALDH1A1, can possibly serve as markers differentiating NOA from obstructive azoospermia (Batruch et al. 2012). The only diagnostic protocol to differentiate between the obstructive azoospermia (OA) and NOA is testicular biopsy. In an attempt to identify markers distinguishing the two, Drabovich et al. identified two proteins, epididymis-expressed ECM1 and testis-expressed TEX101, which differentiated OA and NOA with high specificities and sensitivities (Drabovich et al. 2013). Using DIGE approach, differential protein expression was studied between normal, AS, oligozoospermic, and azoospermic men and found statistically significant increased expression of eight proteins in azoospermia compared with at least one of the other studied groups. The proteins were fibronectin, PAP, proteasome subunit alpha type-3, beta-2-microglobulin, galectin-3-binding protein, PIP, and cytosolic nonspecific dipeptidase, thus providing a deeper insight to the azoospermia condition (Davalieva et al. 2012).

In the search for a panel of common proteins in the fertile males that might be crucial for successful reproduction, a group of investigators performed high-throughput proteomic analysis. Of the 900 proteins resolved, 83 were common in set of five fertile men whose partners conceived 3 months before the study was initiated. Semenogelin I, semenogelin II, olfactory receptor 5R1, lactoferrin, hCAP18,

spindling 1, and clusterin were the common proteins among them suggesting their vigor for reproduction (Milardi et al. 2012). In a recent investigation, differential protein expression of men with abnormal sperm count and sperm morphology was studied (Sharma et al. 2013a). Proteomics analysis revealed 20 differentially expressed proteins among the 4 groups with altered sperm count and abnormal morphology. Among the proteins identified, 3 were downregulated in the group with normal sperm count and abnormal morphology (NA), 1 in oligozoospermia and normal morphology (ON) group, 1 in oligozoospermia and abnormal morphology (OA) group while 2 were upregulated in the ON and OA groups. SP serves as an antioxidant reservoir, antioxidants remove the excess ROS generated in the body thus maintaining a balance and prohibiting oxidative stress. Imbalance in the levels of ROS is reported in SP of infertile men (Wang et al. 2009). Taking this into account, the same researchers studied the molecular mechanisms underlying oxidative stress and sperm dysfunction in infertile men by proteomic profiling. The oxidative stress parameters were assessed (ROS, antioxidant concentration, and DNA damage), and subjects were classified as ROS+ and ROS–. Proteomic analysis revealed 14 proteins in all, with seven proteins common in both the groups. Levels of PIP were elevated in men with increased ROS levels, and gene ontology annotation displayed the extracellular distribution of proteins with a major role in antioxidative activity and regulatory processes (Sharma et al. 2013b).

Similarly, Herwig et al. compared the SP of oligoasthenoteratozoospermia samples with normal fertile males and identified proteins related to oxidative stress, viz., tubulin-folding cofactor B, alpha-1-antichymotrypsin, and aldose reductase (Herwig et al. 2013). Recently, a comparative analysis of oligoasthenozoospermic and normal SP samples revealed that two proteins, namely, epididymal secretory protein E1 and galectin-3-binding protein, were under-expressed in oligoasthenozoospermia and two other proteins, lipocalin-1 and a PIP form, were overexpressed, thus suggesting their involvement in the pathology of idiopathic oligoasthenozoospermic condition (Giacomini et al. 2015).

Conclusion

As a concluding remark, research pertaining to SP proteomics for the search of biomarkers related to specific conditions of male infertility is still ongoing. Integrative approach of proteomic analysis and functional studies annotating the cellular pathways affected has paved the pathway for a deeper insight in mechanisms of male infertility-related pathology. Current research embraces the capability for the development of innovative and clinically relevant male infertility biomarkers using noninvasive procedures, which may provide a better platform for the patients undergoing treatment. Apart from this, success of ART in cases of infertility also needs to be explored. Diverse conditions of infertility have associated with the different sets of proteins. Nevertheless, the data obtained from these studies is heterogeneous as only a small subset of independent studies reporting a small fraction of proteins is found to be overlapping, reason being the use of different proteomic approaches and its combinations. However, the appearance of high-throughput MS-based techniques allows more detailed investigation of the

proteomes of interest, among which is human seminal plasma proteome, and holds promise on more reproducible results in the future. Semen proteomics has the potential to provide information about the regulatory mechanisms of male infertility which is poorly understood till date. The identified proteins should be studied further in deep to find out their exact roles in male infertility. These studies may provide new approaches for management of male infertility.

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